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Fish Oil to Reduce Tobacco Use in Expectant Mothers (FORTUNE) Study

Organization: Vanderbilt University Medical Center

Principal Investigator: Harvey J. Murff, M.D., M.P.H.
Associate Professor
Division of General Internal Medicine and Public Health
6012 Medical Center East
1215 21st Avenue South
Nashville, TN 37212
Phone (615) 936-8319
Fax (615) 936-1269
E-mail: Harvey.j.murff@vanderbilt.edu

Principal Investigator: Hilary A. Tindle, M.D., M.P.H.
Associate Professor
Director, the Vanderbilt Center for Tobacco, Addiction and Lifestyle
Center for Health Behavior & Health Education
2525 West End Avenue, Suite 307
Nashville, TN 37203
Phone (615) 875-9726
Fax (615) 875-2201
E-mail: hilary.tindle@vanderbilt.edu

Investigator: Matthew S. Freiberg, M.D., M.Sc.
Associate Professor
Division of Cardiovascular Medicine
Director, Vanderbilt Translational and Clinical Cardiovascular Research Center
2525 West End Avenue, Suite 300
Nashville, TN 37203
Phone (615) 875-9729
E-mail: matthew.s.freiberg@vanderbilt.edu

Investigator: Katherine E. Hartmann, M.D., Ph.D.
Associate Dean for Clinical and Translational Scientist Development
Deputy Director, Institute for Medicine and Public Health
Director, Women's Health Research
2525 West End Avenue, Suite 600
Nashville, TN 37203
Phone (615) 322-4785
Fax (615) 936-8291
E-mail: Katherine.hartmann@vanderbilt.edu

Investigator: Rosette J Chakkalakal, M.D., M.P.H.
Assistant Professor
Division of General Internal Medicine and Public Health
6000 Medical Center East
1215 21st Avenue South
Nashville, TN 37212
Phone (615) 936-2187
Fax (615) 936-3218
E-mail: rosette.j.chakkalakal@vanderbilt.edu

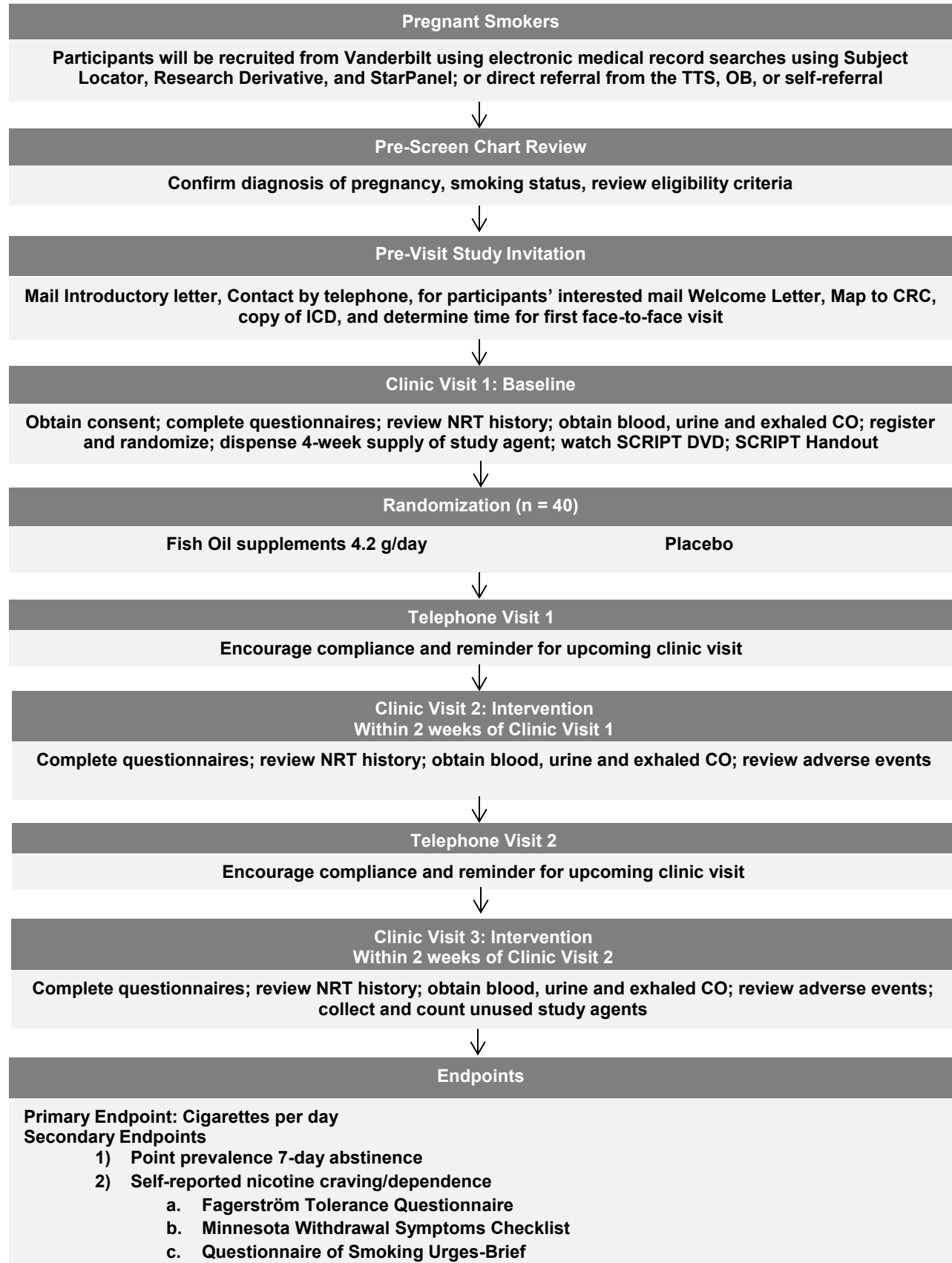
Investigator: Qiuyin Cai, M.D., Ph.D.
Associate Professor of Medicine
Division of Epidemiology
Director, Molecular Epidemiology Biospecimen Core
1161 21st Avenue South, MCN B-2104
Nashville, TN 37232
Phone (615) 936-1351
Fax (615) 322-1254
E-mail: qiuyin.cai@vanderbilt.edu

Statistician: Robert A. Greevy, Ph.D.
Associate Professor
Director, Health Services Research Biostatistics
Department of Biostatistics
2525 West End, Suite 11000
Nashville, TN 37203
Phone (615) 343-5793
Fax (615) 343-4924
E-mail: robert.greevy@vanderbilt.edu

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1.0 Rationale and Specific Aims

The purpose of this feasibility study is to obtain pilot data in preparation for an upcoming R01 submission (June 2017). The goals of that submission will be to conduct a clinical trial of n-3 LCPUFAs for smoking cessation in pregnant women. For this VICTR proposal, we will develop, test, and refine our recruitment strategy, and collect data demonstrating our ability to successfully recruit pregnant women who are actively smoking. We will collect side effect, tolerability, and adherence data regarding our intervention. Finally, we hope to gather preliminary effect size data to allow more formal estimates of sample size. *We hypothesize that pregnant smokers randomized to n-3 LCPUFA supplementation will have higher smoking cessation rates and less nicotine cravings compared to women allocated to placebo.* We intend to use this preliminary data to inform a future randomized, double-blind, placebo-controlled trial of n-3 LCPUFA supplementation for tobacco cessation.

Our specific aims are:

SA1: To successfully recruit and randomize 40 pregnant, current smokers into a clinical trial of n-3 LCPUFA supplementation.

SA2: To assess the tolerability of 4 gm/day n-3 LCPUFA supplementation in pregnant, current smokers over a 4-week period

SA3: To determine the effect of 4 grams/day n-3 LCPUFA supplementation for 4-weeks on smoking cessation rates, cigarettes smoked per day, and self-reported nicotine cravings in pregnant smokers

2.0 Background

Cigarette smoking is the leading cause of preventable mortality and quitting smoking significantly reduces the risk of tobacco-related diseases (1). Smoking is considered the most important modifiable risk factor for adverse pregnancy outcomes and is associated with preterm deliveries, growth restriction of infants, pre-term related deaths, and sudden infant death syndrome (2-5). It is estimated that almost 11% of American women report smoking during pregnancy with higher rates in younger women with lower educational levels (6, 7). Rates of quitting smoking during pregnancy have been reported ranging from 35-75% with risk factors for lack of quitting including lower educational status, smoking 10 or more cigarettes a day, multiparity, and coexisting psychiatric problems (6-8). For these particular high-risk individuals promoting smoking cessation can be challenging given concerns regarding both the safety and efficacy of currently FDA-approved pharmacologic treatment in pregnancy and breast feeding women. As such, currently there are limited pharmacologic approaches for smoking cessation in this high risk group of pregnant women. The identification of safe and effective adjuvant therapies to promote smoking cessation in pregnant women would have a powerful clinical impact on maternal-fetal health outcomes.

n-3 PUFAs have anti-inflammatory effects and are possibly cardio-protective (9). We and others have found that red blood cell phospholipid (RBC) membrane concentrations of long-chain n-3 (LC) PUFAs (EPA and DHA) are significantly reduced in smokers compared to non-smokers (10-14). The mechanisms behind this relative n-3 LCPUFA deficiency are not known, however, possible reasons include 1) dietary differences; 2) preferential lipid peroxidation of n-3 PUFA versus n-6 PUFAs; and 3) tobacco-related changes in endogenous n-3 LCPUFA metabolism.

Emerging data is beginning to suggest that n-3 LCPUFA relative deficiencies could have a role in behavior. In animal models, n-3 PUFA deficiencies result in structural changes in nervous tissue which impacts dopaminergic and serotonergic systems and correction of these deficiencies can reverse these changes (15-18). In particular, n-3 PUFA deficiency can result in hypofunctioning of the dopamine mesocorticolimbic pathways which are related to reward and dependence (19, 20). Nicotine results in an elevation of dopamine in the nucleus accumbens which is associated with the pleasurable sensations related to nicotine use (21, 22). As such, it has been hypothesized that correcting the hypofunctioning dopaminergic system through n-3

LCPUFA supplementation might reduce the symptoms of withdrawal associated with smoking cessation and reduce nicotine cravings (23).

Recently, two small scale, double-blind, randomized control trials have found that supplementation with n-3 LCPUFA reduces signs of nicotine dependence (24, 25). It is important to note that neither of these studies recruited subjects who wished to quit smoking and at no time during either study was smoking cessation encouraged. Rabinovitz randomized 50 healthy smokers to either 4750 mg of EPA + DHA per day for 30 days or placebo (24). The study reported only 1 subject loss to follow-up and found a statistically significant reduction in self-reported tobacco craving and cigarettes per day in n-3 LCPUFA group compared to the placebo group. Of interest, even 30 days after stopping the intervention, individuals allocated to the n-3 LCPUFA group reported lower levels of tobacco cravings compared to the placebo group. Zapparoli et al randomized 63 healthy-smokers to either 1023 mg/d EPA + DHA or placebo for 90 days(25). Loss of follow-up was considerable in this study with only 62% of the participants completing the study (38% drop out). The study was analyzed using an intention-to-treat design. A statistically significant difference was seen in nicotine dependence between the n-3 PUFA and placebo group with a 23% reduction in dependence score for smokers allocated to n-3 LCPUFA. However, there was no difference between the groups in cigarettes per day. Reasons for the discrepancy between the two trials could be related to the dose of n-3 PUFA chosen and the rate of subject drop-out. **Taken together, these studies suggest that supplemental n-3 LCPUFA might be useful in promoting smoking cessation. In addition, given that these trials were conducted in individuals who were not interested in quitting smoking, perhaps even greater effects might be seen in individuals who wish to stop smoking.**

Because the n-3 LCPUFA DHA has such an important role in fetal neural development there has been significant interest in the use of fish oil supplements in pregnancy. A meta-analysis of 11 randomized trials of fish oil supplementation versus no supplementation found no differences in cognitive, language or motor development between the intervention and control arms. Of note, only two of these studies provided above 2 grams per day of n-3 LCPUFA, possible under dosing the intervention (26). In general most interventional studies have included few smokers and smoking status was only assessed at baseline (27). Thus it is unclear how adequately dosed n-3 LCPUFAs might impact tobacco use in pregnant women who smoke. Nevertheless, even larger doses of fish oil supplementations are well tolerated, with the most common side effect reported being fishy “burps” with no differences in bleeding complications, nausea, emesis, diarrhea, or abdominal pain (28). Although several expert panels recommend pregnant and lactating women to consume at least 200 to 300 mg/day of DHA on average, no formal guidelines exist advocating the use of supplements (29, 30).

3.0 Summary of Study Plan

Design

Eligible patients will be pregnant women who are currently smoking seen at Vanderbilt University Medical Center. The study is a single-site, randomized, double-blind, placebo-controlled, parallel arm trial design. The two arms included one treatment arm and one placebo arm. Treatment will be 4.2 grams/day of EPA + DHA. Placebo will be 5 grams of olive oil in capsule form. The intervention period will last 4-weeks. We will randomize 40 participants into the two treatment arms. As this is a feasibility study, we have not powered the study to account for drop-outs.

Recruitment and Screening

Participants will be identified through searching Subject Locator, queries of the Research Derivative, direct referral from the Tobacco Treatment Service, or self-referral from clinic fliers. Study physicians will conduct a medical chart review and participants who are potentially eligible will be invited for Clinic Visit 1 (baseline).

Clinic Visits

Clinic Visit 1: After eligibility is confirmed, consent will be obtained. A brief medical history will be obtained. Participants will complete four questionnaires. Questionnaires will be administered by study staff or CRC nurses directly into REDCap study databases. Blood, urine, and exhaled CO measurements will be collected. Participants will be dispensed a four-week supply of medication. Participants will be given a pill diary.

Clinic Visit 2: Participants will complete study questionnaires. Blood, urine, and exhaled CO measurements will be collected. Participants will report any adverse events.

Clinic Visit 3: Participants will complete study questionnaires. Blood, urine, and exhaled CO measurements will be collected. Participants will report any adverse events and return any unused medications.

4.0 Participant Selection

4.1 Inclusion Criteria

4.1.1 ≥ 18 or ≤ 45 years of age

4.1.2 Currently reporting daily cigarette use (≥ 1 CPD, no averages must have daily use)

4.1.3 Between 6 and 36 weeks gestation

4.2 Exclusion Criteria

4.2.1 Allergy to fish or seafood or Lovaza

4.2.2 Currently using fish oil supplements and unwilling to stop prior to and during the trial

4.2.3 Unstable psychiatric disease: Defined as requiring hospitalization or active medication changes (medication changes or up titration) within the preceding 3 months

4.2.4 Unstable pregnancy-related medical problems (pre-eclampsia, premature labor, threatened abortion, oligohydramnios, placenta previa, hyperemesis gravidarum, HELLP syndrome, , cholestasis of pregnancy, , , placenta accreta)

4.3 Inclusion of Women and Minorities

The study will only recruit women which are justified scientifically. Members of all races and ethnic groups are eligible for the trial.

4.4 Recruitment and Retention Plan

Recruitment into the study will follow a six step process including: 1) identification of potential participants through electronic prescreening to identify potentially eligible candidates by querying Subject Locator, Research Derivative, and StarPanel **OR** direct referral from the TTS **OR** OB or self-referral; 2) medical record review for a preliminary check of eligibility criteria; 3) initial participant contact first via an introductory letter and then via telephone contact to describe the study, determine interest, and arrange for a face-to-face visit; 4) contacting the CRC to schedule the visit; and 5) mailing the introductory packet.

4.4.1 Identification of potential participants

4.4.1.1 Electronic prescreening

Vanderbilt University Medical Center has invested heavily in infrastructure to recruit subjects for clinical trials and we will utilize all of our potential resources. Potentially eligible participants will be identified through multiple sources.

We will utilize SUBJECT LOCATOR as a recruitment tool to identify potential research subjects based on data available in VU clinical systems (e.g. STAR Panel, WizOrder, Clinic Scheduling). The SUBJECT LOCATOR

program is part of a toolset available through VICTR that enables teams to specify inclusion/exclusion criteria for a specific study. The inclusion/exclusion criteria are codified for computable use and combined with data coming through VU Clinical Systems to proactively identify individuals who might qualify for a study. Once a 'match' is made, research study personnel are alerted using confidential messaging or a secure web portal.

We will identify pregnant women by querying upcoming obstetrics visits occurring within the next 28 days as potential subjects. We will identify smokers by querying as free text searches for the terms “cigarettes”, “cigarettes/day”, “pack/day”, “ppd” within the problem list and clinical notes. We will also search using ICD-10 codes for tobacco use disorders (listed below).

Search algorithm for SUBJECT LOCATOR:

Set Details:

- 1) Select 27 clinics associated with obstetrics/midwifery/MFM
- 2) 28 day range to scan

Inc/Exc Criteria Details

Identifies Smokers

- 1) Keyword ‘cigarettes per day’ in PL [OR]
- 2) Keyword ‘cigarettes’ in PL [OR]
- 3) Keyword ‘cigarettes/day’ in PL [OR]
- 4) Keyword ‘packs per day’ in PL [OR]
- 5) Keyword ‘packyears’ in PL [OR]
- 6) Keyword ‘pack years’ in PL [OR]
- 7) Keyword ‘PPD’ in PL [OR]
- 8) ICD10 code O99.331 [OR]
- 9) ICD10 code O99.332 [OR]
- 10) ICD10 code O99.333 [OR]
- 11) ICD10 code Z71.6 [OR]
- 12) ICD10 code Z72.0 [OR]
- 13) ICD10 code F17.293 [OR]
- 14) ICD10 code O99.330

Search algorithm for RESEARCH DERIVATIVE:

The Research Derivative is a database of clinical and related data derived from the Medical Center's clinical systems and restructured for research. Data is repurposed from VU's enterprise data warehouse, which includes data from StarPanel, VPIMS, and ORMIS (Operating Room Management Information System), EPIC, Medipac, and HEO among others. The medical record number and other person identifiers are preserved within the database. Data types include reimbursement codes, clinical notes and documentation, nursing records, medication data, laboratory data, encounter and visit data, among others. Output may include structured data points, such as ICD 9 codes or encounter dates, semi-structured data such as laboratory tests and results, or unstructured data such as physician progress reports. The database is maintained by the Office of Research Informatics under the direction of Paul Harris, Ph.D.

Searches of RD will include identifying individuals' ages 18 to 45 years of age, with a B-HCG measured within the past 180 days AND with a value ≥ 1000 . RD will be searched to identify individuals with upcoming clinic visits (any clinic). Smoking status will be queried using the RAN/CIF forms. Information that will be captured and transmitted to Dr. Murff via a secure file transfer will include: name, MRN, phone number.

4.4.1.2 Direct referral from the TTS

In addition, in conjunction with the ongoing inpatient Tobacco Treatment Service in VUH, Dr. Tindle and the TTS are partnering with Obstetrics providers, whose support we are enlisting to begin the process of smoking cessation treatment as early as possible in the pregnancy. If the TTS identifies a potentially eligible participant in performing their clinical duties they will contact the Dr. Murff by either pager/cell (615 835-9617 pager/615 969-0425 cell) or secure email (Harvey.j.murff@vanderbilt.edu).

4.4.1.3 Participant self-referral or direct OB referral

Fliers with study contact information will be placed in VUMC obstetric clinics. Participants who contact study investigators indicating an interest in participating will be considered potential participants. We will also meet with OB clinicians and clinical leaders to describe the protocol. OB's or midwives may directly refer to Dr. Murff by either pager/cell (615 835-9617 pager/615 969-0425 cell) or secure email (Harvey.j.murff@vanderbilt.edu).

During the identification of potential participants, study personnel will initiate a subject as a potential participant adding identified individuals to an Excel database. The database will be called the **Master Eligible File**. The choice of the Excel databases is to allow for the immediate destruction of the PHI on potential participants who were not eligible for the study. The form will be utilized to ensure records for potential subjects are not reviewed twice or a potential subject who was not interested in participating is not contacted twice. The Excel database will document:

1. Participant Name
2. Medical Record Number
3. Entry Date
4. Medical Record Review Complete (Y/N)
5. Eligible (Y/N)
6. Telephone Number (Only recorded for potentially eligible individuals)
7. Verbal Consent obtained (Y/N)

4.4.2 Medical record review

Study physicians will access the Master Eligible File on a regular basis to identify potential participants for medical record review. We will conduct a preliminary medical record review to confirm inclusion and exclusion criteria (**Appendix A: Eligibility Checklist**).

4.4.3 Initial participant contact

Once a potential participant has been identified an introductory letter will be mailed. After 1 week after the letter is mailed subjects will be contacted prior to their visits to determine 1) current smoking status, 2) eligibility status, and 3) interest in participating (**Appendix E: Initial Contact Telephone Script**). Subjects who note that they are interested in participating will have their current address and contact information verified, will be asked to recommend several potential dates within the next 2 weeks for the first in person visit. They will be told that they will be mailed a study introductions packet (**Appendix D: Study Introductory Packet**) and will be called to confirm the appointment (**Appendix F: Appointment Confirmation Contact**). Subjects who give verbal consent will be instructed to stop any over-the-counter fish oil supplementations. Subjects who are interested to participate will be entered into the Tracker REDCap database. Contact information will be verified and updated and the following information will be added:

1. Telephone Interviewers Initials
2. Telephone Contact Date
3. Participant Name
4. FORTUNE Study ID Number

5. Current Contact Information
6. Preferred means of communication (phone/email)
7. Email address
8. Preferred number
9. Permission to leave messages
10. Inclusion and Exclusion Criteria
11. Current Smoking Status
12. Requested First Visit Dates

For all telephone procedures attempts will be made to telephone each participant for the purposes of recruitment. For appointments and reminders a message may be left on an answering machine or with a household member to call regarding study (**Appendix C: Telephone/Email Reminder Scripts**). For telephone calls requiring direct participant contact if greater than 15 attempts to contact the potential participant have been unsuccessful then the participant will be excluded from study participation

Potential participants who are not interested in participating or do not meet study inclusion or exclusion criteria will be considered not eligible and no further contact is required.

4.4.4 Contacting the CRC

Study personnel will schedule the CRC visit time using the online CRC reservation system. After the reservation is confirmed and reminder call will be made to the subjects to notify them of their visit. The Tracker REDCap database will be updated with the following information

13. Scheduled CRC Date
14. Reminder Call
15. Date of Completion

4.4.5 Mailing the introductory packet

Study personnel will mail a study introductions packet (**Appendix D: Study Introductory Packet**) which will include 1) welcome letter, 2) map to the CRC, and 3) a copy of the informed consent document to the potential subject. The Tracker REDCap database will be updated with the following information

16. Date Introductory Packet Mailed

5.0 Agent Administration

Intervention will be administered on an outpatient basis. Reported AE's and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

The whole intervention period lasts for 4 weeks (range: 3 weeks 5 days – 4 weeks and 2 days):

Control Arm: Placebo Olive oil supplement capsules 5 grams/daily in five (5) daily capsules (n=20)

Experimental Arm: Lovaza® 4.2 grams/daily in five (5) daily capsules (n = 20)

5.2 N-3 LCPUFA Administration

Lovaza® is purchased from GlaxoSmithKline (Research Triangle Park, NC) and placebo is purchased from Swanson Health Products (Fargo, ND). The study PI will purchase placebo. The Vanderbilt Investigational Drug Service (IDS) will purchase Lovaza®. The Vanderbilt IDS will be responsible for the storage (controlled

room temperature 59° F – 86 ° F and under low humidity and low light), preparation of all investigational agents, and maintenance of accurate drug storage and dispensing logs.

Participants allocated to fish oil supplementation will be instructed to take five Lovaza® capsules each containing 465 mg of EPA and 375 mg DHA daily; this will provide a total daily dose of 2325 mg EPA plus 1875 mg DHA for a total daily dose of fish oil of 4.2 grams.

Rationale for Dose

As described above, two prior studies have been conducted in smokers to assess the effects of supplemental n-3 LCPUFAs on nicotine cravings (24, 25). While both studies reported a statistically significant reduction in self-reported nicotine cravings in the intervention arms, a reduction in cigarettes smoked per day was only seen in the study of high-dose n-3 LCPUFA (> 4 grams/day) as opposed to low dose n-3 LCPUFA (1 gram/day). These studies would suggest a dose effect and as such we are using a high-dose n-3 LCPUFA intervention for this pilot study.

Each participant will receive three bottles given at a single dispensing time (Visit 1):

- Bottle 1-3 labelled “Weeks 1-4” (154 capsules).

Bottles 1-3 will be dispensed at Clinic Visit 1 (Baseline Visit). The prescription (**Appendix B: Visit Materials**) and the signed consent document (for Clinic Visit 1 only) will be faxed to the Vanderbilt IDS. The Vanderbilt IDS will transport the capsules to the CRC unit. Either the CRC nurse or study staff will give the bottles to the participant. Participants will be instructed to take the study medication with food to decrease the risk of GI upset and to store the medication in a refrigerator. Refrigeration of the product is to reduce the risk of eructation and “fishy” taste in the mouth. They will be instructed not to freeze the capsules. To enhance tolerability subject will be given the following dose-escalation schedule.

- Day 1: Take 2 capsules
- Day 2: Take 3 capsules
- Day 3: Take 4 capsules
- Day 4: Take 5 capsules

Participants are allowed to take all 5 capsules at one time or in divided doses. Participants will be advised to maintain the same dosing pattern over the course of the study to improve compliance. Participants will be given a pill diary as a visual memory aide at Clinic Visits 1 (**Appendix B: Visit Materials**). Between Clinic Visits 1 to 2 and 2 to 3, subjects will receive a single telephone reminder to encourage medication compliance using a standardized telephone script (**Appendix C: Telephone/Email Reminder Scripts**). This message can be left either on a voice mail or with a different household member if the participant allows. In addition, alternative message options (email communications) will be allowed for medication and visit reminders if the participant indicates these as their preferred method of communication. Standardized telephone and email reminders are in **Appendix C: Telephone/Email Reminder Scripts**.

If the participant is unable to stay at the clinic to receive the capsules or run out of study medication before the final visit can be scheduled, the pharmacist at the Vanderbilt IDS will mail the capsules via FedEx within one business day of the Clinic Visit. Research staff will follow-up with a phone call to the participant to ensure receipt of the study agent.

5.3 Run-in Procedures

Not applicable

5.4 Contraindications

Lovaza® is contraindicated in participants with a known hypersensitivity to Lovaza® or any of its components.

5.5 Concomitant Medications

There are no restricted concomitant medications.

5.6 Dose Modification

Individuals who report any GI symptoms \geq grade 3, such as dyspepsia, heartburn, or diarrhea, will be instructed to discontinue the study medication immediately.

Participants reporting minor GI symptoms (\leq grade 2) will be assessed to ensure they are taking the medication as suggested (with meals and refrigerated). Those who wish to remain on trial will be instructed to reduce their study medication from 5 capsules daily to 4 capsules daily. If symptoms persist or worsen over the next 3 days they will be instructed to reduce their daily dose from 4 capsules per day to 3 capsules per day. If symptoms persist or worsen over the next 3 days they will be instructed to reduce their daily dose from 3 capsules per day to 2 capsules per day. If symptoms persist or worsen over the next 3 days they will be instructed to discontinue the study medication immediately.

5.7 Adherence/Compliance

Unused medications will be collected at Clinic Visits 2 and 3. Participants will be considered evaluable if they have taken 70% of the prescribed dose and/or have a 100% increase in RBC phospholipid membrane total n-3 LCPUFA (EPA + DHA) concentration from baseline to 4 weeks.

Participant compliance will be monitored in a variety of ways. Returned pills will be counted, pill diaries collected, and RBC phospholipid membrane n-3 LCPUFA content monitored.

6.0 Pharmaceutical Information

6.1 Lovaza®

Lovaza® is a combination of ethyl esters of omega3 fatty acids, principally EPA and DHA. Lovaza® capsules are supplied as 1-gram transparent soft-gelatin capsules filled with light-yellow oil. Each 1-gram capsule of Lovaza® contains at least 900 mg of the ethyl esters of omega-3 fatty acids sourced from fish oils. These are predominately a combination of eicosapentaenoic acid (approximately 465 mg) and docosahexaenoic acid (approximately 375). Lovaza® capsules also contain the following inactive ingredients: 4 mg α -tocopherol (in a carrier of soybean oil) and gelatin, glycerol, and purified water.

6.2 Placebo

Placebo will be Swanson® Brand Essential Fatty Acids Olive Oil Supplement. Capsules are supplied as 1-gram transparent soft-gelatin capsules filled with light-yellow oil. Each 1-gram capsule contained 1000 mg of extra-virgin olive oil. Other ingredients include gelatin, glycerin, and purified water.

We will use oleic acid as our placebo. The reason for the use of oleic acid is several-fold. First, oleic acid (olive oil) capsules have a similar texture, size, color, and consistency to fish oil capsules. Oleic acid has been used as a placebo in several prior studies and is well tolerated.(31-33)

6.3 Reported Adverse Events and Potential Risks

The most common adverse effects associated with Lovaza® include: eructation, dyspepsia, and taste perversion (incidence $>$ 3% and greater than placebo). Other GI side effects reported include: diarrhea, nausea, abdominal pain, abdominal distention, constipation, and vomiting. Adverse events will be collected on the AE CFR (**Appendix G: Adverse Event Form**).

6.4 Packaging/Labeling and Storage

The study agents will be packaged and labelled by the Vanderbilt IDS. One bottle will be dispensed at Clinic Visit 1 and a second bottle at Clinic Visit 2. Each bottle will contain either 74 or 80 capsules. The Vanderbilt IDS will be responsible for the storage (controlled room temperature 59° F – 86 ° F and under low humidity and low light), preparation of all investigational agents, and to maintain accurate drug storage and dispensing logs.

6.5 Randomization

This study will recruit 40 participants who are currently pregnant and actively smoking. A 1:1 permuted block randomization design stratified based on current tobacco use (≤ 5 cigarettes per day or > 5 cigarettes per day) will be used to allocate the participants to the intervention or placebo arm and will be created by the study statistician. The randomization schema will be transmitted to the IDS. Both subjects and study staff will be blinded to the assignments.

Procedures:

1. Participants will review and sign an informed consent document
2. The signed ICD and prescription for the study medication will be faxed to the IDS
3. The IDS will randomize the participant based on the study statistician schema
4. The IDS will transmit the study medication to study personnel in the CRC

6.6 Blinding and Unblinding Methods

The research pharmacist will manage the investigational agent. The blind will be maintained through the effort of the research pharmacist and the pharmacy. Unblinding will only occur when it is deemed medically necessary, and will only take place after consultation with all study investigators. The date and the reason for breaking the blind must be recorded.

6.7 Agent Destruction/Disposal

After completion of investigation, all unused study agent will be returned to the Vanderbilt IDS for destruction/disposal.

7.0 Clinical Evaluations and Procedures

7.1 Schedule of Events

	<u>Prescreening:</u> -2 weeks	<u>Clinic Visit 1:</u> Baseline: 0 weeks	<u>Telephone</u> <u>Visit 1:</u> ~1 week of Baseline ¹	<u>Clinic Visit 2:</u> ~2 week of Baseline	<u>Telephone</u> <u>Visit 2:</u> ~1 week of Clinic Visit 2	<u>Clinic Visit 3:</u> ~2 week of Clinic Visit 2
Recruitment	X					
Eligibility Assessments	X	X				
Informed Consent		X				
Randomization		X				
Questionnaires: Clinic Visit 1: FTND, MNWS, PHQ-4, QSU-brief, Tobacco Use questionnaire, Baseline Visit questionnaire.		X		X		X

Clinic Visits 2 & 3: FTND, MNWS, PHQ-4, QSU-brief, Tobacco Use questionnaire						
Behavioral Intervention		X				
Exhaled CO		X		X		X
RBC fatty acids		X		X		X
Serum NMR		X				
Blood for Storage		X				X
Oral flora collection		X		X		X
Med Dispense		X				
Urine Collection		X		X		X
Collect Unused Pills						X
Review Med Diary						X
Compliance Reminder			X	X	X	X
Assess Adverse Event				X		X
Reimbursement		X		X		X

¹ = ± 2 days

7.2 Pre-study Evaluation

Prescreening Period:

- Research staff will use electronic searches and direct clinical referrals to identify pregnant smokers.
- The study physician will conduct a chart review to confirm eligibility and obtain contact information
- Introductory letters will be sent 1 week prior to telephone calls
- Research staff will contact potential participant to describe the study, evaluate eligibility, and determine if they are interested
- Interested participants will be scheduled for Clinic Visit 1 at the CRC
- An Introductory packet of materials will be mailed to the potential participant

7.3 Evaluation During Study Intervention

Clinic Visit 1 (Baseline)

- Review inclusion and exclusion criteria
- Obtain informed consent
- Subjects completes
 - Fagerström Test for Nicotine Dependence (FTND) questionnaire
 - Minnesota Nicotine Withdrawal Symptom Checklist (MNWS)
 - PHQ-4
 - Questionnaire of Smoking Urges (QSU)-brief
 - Tobacco use questionnaire
 - Baseline Visit Questionnaire/Baseline GI Symptoms
- Obtain the following specimens
 - Expired CO level
 - Urine sample
 - Collection of oral flora
 - Three tubes of blood collection
 - Red blood cell phospholipid membrane
 - Nicotine metabolites (serum cotinine and 3-hydroxycotinine)
 - Blood for banking.
- Randomization
- Fax prescription to IDS
- Dispense 4-week supply of study medication
- Subject receives
 - Pill Diary
- Watch SCRIPT DVD and dispense SCRIPT materials

- Schedule Week 2 and Week 4 visits

Clinic Visit 2 (Mid-Study)

- Subjects completes
 - Fagerström Test for Nicotine Dependence (FTND) questionnaire
 - Minnesota Nicotine Withdrawal Symptom Checklist (MNWS)
 - PHQ-4
 - Questionnaire of Smoking Urges (QSU)--brief
 - Tobacco use questionnaire
- Obtain the following specimens
 - Expired CO level
 - Urine Collection
 - Collection of oral flora
 - Blood collection
 - Red blood cell phospholipid membrane
- Interview subjects for any signs of adverse events

Between-Visits Telephone Contacts

Weeks 1 and 3

Participants will be contacted in Weeks 1 and 3 via telephone to

- Encourage medication compliance
- Remind subject regarding upcoming appointment

Based on participants' preference, these communications can also be conducted via electronic mail.

7.4 Evaluation at Completion of Study Intervention

Clinic Visit 3 (End-Visit)

- Subjects complete
 - Fagerström Test for Nicotine Dependence (FTND) questionnaire
 - Minnesota Nicotine Withdrawal Symptom Checklist (MNWS)
 - PHQ-4
 - Questionnaire of Smoking Urges (QSU)-brief
 - Tobacco use questionnaire
- Obtain the following specimens
 - Expired CO level
 - Urine sample
 - Collection of oral flora
 - Blood collection
 - Red blood cell phospholipid membrane
 - Blood for banking
- Collect unused pills to conduct pill counts
- Collect pill diary
- Interview subjects for any signs of adverse events
- Participant is asked to speculate on what therapy they were randomized

To enhance subject retention and offset travel costs we will reimburse subjects \$40 USD per clinic visit for a total over the course of the study of \$120.00.

7.5 Post-Intervention Follow-up Period

As part of this feasibility study there is no post-intervention follow-up planned

7.6 Methods for Clinical Procedures

Blood Sample Collection Procedure

Blood samples will be collected at all Clinic Visits. Blood will be drawn into one EDTA tube (all 3 visits), one heparin tube for plasma (visit 1 only), and one red top tube for long term storage (visit 1 and visit 3 only).

End-Exhaled CO Procedure

Participants will be advised to hold their breath for 15 seconds before blowing in the device. The results of the device will be recorded into the REDCap database.

Collection of Oral Flora

Participants will be advised swish 1-2 tablespoons of Scope (or generic equivalent) in their mouth for 5 seconds and then spit into a specimen collection container. This sample will be transported to the Molecular Epidemiology Laboratory.

8.0 Criteria for Evaluation and Endpoint Determination

8.1 Primary Endpoint

The primary outcome will be reduction in total number of cigarettes per day from baseline to 4-weeks.

8.2 Secondary Endpoint

The secondary outcomes will include: 1) reduction in the Fagerström Tolerance Questionnaire and 2) point prevalence abstinence at 4 weeks biochemically confirmed by end-expired carbon monoxide.

Safety and tolerability outcomes will include: 1) reported AEs and 2) the rate of discontinuation of study medication due to side effects.

8.3 Off-Agent Criteria

Participants may stop taking the study agent for the following reasons:

- 1) Individuals who reports minor GI symptoms such as dyspepsia, heartburn or diarrhea who do not wish to remain on trial
- 2) Noncompliance with study agent
- 3) Medical contraindication

Participants who go off-agent will be withdrawn from the study

8.4 Off-Study Criteria

Accrual will be stopped if at any time toxicity among participants requires withdrawal of 25% of participants based on toxicity criteria outlined in Section 5.6.

8.5 Study Termination

The study PI(s) has the right to discontinue the study at any time.

9.0 Specimen Management

9.1 Laboratories

RBC phospholipid membrane fatty acid analyses will be conducted by the Hormone Assay and Analytical Services Core Lab-Lipid Lab (Dr. Larry Swift, Director).

Nicotine and tobacco metabolites will be determined using the VUMC Clinical Laboratories (ARUP).

Blood specimens will be processed within the CRC and stored in the -80°C freezers in the Molecular Epidemiology Biospecimen Laboratory (Dr. Qiuyin Cai, Director).

Oral microbiome specimens will be processed in the Molecular Epidemiology Biospecimen Laboratory (Dr. Qiuyin Cai, Director).

9.2 Collection and Handling Procedures

End-Expired CO levels

We will use the piCO Smokerlyzer (Bedfont Instruments) to assess end-expired carbon monoxide. Participants will be advised to hold their breath for 15 seconds before blowing in the device. Output is in CO ppm and hb%. Subjects self-reporting abstinence may have smoking status confirmed biochemically using end expired carbon monoxide. Biochemically validated abstinence will be defined as end expired carbon monoxide less than 10 ppm.

Serum Cotinine and 3-hydroxycotinine

Nicotine metabolites will be measured using an isotope dilution-high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry using a commercially available testing center. The assay requires 1.5 ml of serum. Serum cotinine has an advantage over end-expired CO as it reflects a greater period of abstinence detecting nicotine exposure for up to 14 days as opposed to 12-24 hours for expired CO.

Urine nicotine and metabolites testing

Nicotine metabolites will be measured using a quantitative liquid chromatography-tandem mass spectrometry using a commercially available testing center. The assay requires 4 ml of urine. The assay measures 3-hydroxycotinine, anabasine, cotinine, nicotine and nornicotine.

Determination of RBC Phospholipid Fatty Acid Analysis

Lipids will be extracted using the method of Folch-Lees (113). Fatty acid methyl esters are identified by comparing the retention times to those of known standards. Inclusion of the internal standard, dipentadecanoyl phosphatidylcholine (C15:0), permits quantitation of phospholipid amount in the sample.

9.3 Specimen Storage

Protocol for blood sample collection

We will collect a total of 24 ml non-fasting blood on each subject to be drawn into a serum, heparin, and EDTA. Blood samples will be collected at study baseline (week 0) (12 ml-three tubes), the study midpoint (week 2) (4ml-one tube) and the end of the study (week 4) (8 ml-two tubes). Collected blood will be used for the following study related measures:

- Erythrocyte phospholipid fatty acid content: This will be a marker of medication compliance.
 - Tube type: EDTA lavender top
 - Will collect on all three visits
 - Blood volume required: 200µL RBCs
 - Specimen processing: see below
 - Laboratory location for storage:
 - Molecular Epidemiology Specimen Laboratory
MCN, B-2104, ext # 6-1351

- Laboratory for specimen processing:
 - CRC Specimen Laboratory
MCN, Suite AA-3208
- Laboratory for fatty acid measurement:
 - Swift Laboratory
CC3327 Medical Center North
- Serum nicotine metabolites: This will be a marker of exposure assessment
 - Tube type: green top tube
 - Will collect at baseline only
 - Blood volume required 3ml
 - Specimen processing: see below
 - Laboratory location for storage:
 - Molecular Epidemiology Specimen Laboratory
MCN, B-2104, ext # 6-1351
 - Laboratory for specimen processing:
 - CRC Specimen Laboratory
MCN, Suite AA-3208
 - Laboratory for serum nicotine metabolites:
 - TVC laboratories
- Blood for storage: This will be banked for future studies
 - Tube type: red top tube
 - Will collect at baseline and week 4
 - Blood volume required 4ml
 - Specimen processing: see below
 - Laboratory location for storage:
 - Molecular Epidemiology Specimen Laboratory
MCN, B-2104, ext # 6-1351

Required Phlebotomy Materials:

- Tourniquet
- Non-latex disposable gloves
- Evacuated Tubes
- 70% alcohol pads
- 20 or 21 Gauge butterfly needle for the forearm or 25 Gauge needle for the wrist or hand
- Red, Lavender and Green top collection tubes
- Specimen Labels
- Specimen Bags
- 2 x 2 Gauze pads
- Adhesive Tape or Bandages

Procedure

- Wash your hands thoroughly and don non-latex disposable gloves to prevent cross-contamination.
- Ask the patient to sit in a chair and support his arm securely on an armrest or tabletop.
- Assess the patient's veins to determine the best puncture site. Observe the skin for the vein's blue color, or palpate the vein for a firm rebound sensation.
- Tie a tourniquet 2" proximal to the area chosen. If the tourniquet fails to dilate the vein, have the patient open and close his fist repeatedly. Then ask him to close his fist as you insert the needle and to open it again when the needle is in place.
- Clean the venipuncture site with an alcohol pad using friction for 30 seconds. Wipe in a circular motion, spiraling outward from the site. Allow to dry before performing venipuncture.

- Immobilize the vein by pressing just below the venipuncture site with your thumb and drawing the skin taut.
- Position the syringe with the needle bevel up and the shaft parallel to the path of the vein and at a 30-degree angle to the arm. Insert the needle into the vein. Grasp the needle holder securely to stabilize the vein, and push down on the collection tube until the needle punctures the rubber stopper. Blood will flow into the collection tube automatically.
- Remove the tourniquet as soon as the blood flows adequately to prevent stasis and hem concentration, which can impair test results. If blood flow is sluggish, leave the tourniquet in place longer, but always remove it before withdrawing the needle.
- Continue to fill the required tubes, removing one and inserting another. Gently rotate each tube as you remove it to help mix the additive. Order of fill is lavender/green/red
- After you've drawn the sample, place a gauze pad over the puncture site, and slowly and gently remove the needle from the vein. When using an evacuated tube, remove it from the needle holder to release the vacuum before withdrawing the needle from the vein.
- Apply gentle pressure to the puncture site for 2 to 3 minutes or until bleeding stops. This prevents extravasation into the surrounding tissue, which causes hematoma.
- After the bleeding stops, apply an adhesive bandage.
- Label the patient chart with appropriate blood tube labels and record the time of collection on sample collection form.
- Place the tubes into black wet ice bucket.
- Finally, check the venipuncture site to make sure a hematoma has not developed. If it has, then apply warm soaks.
- Discard syringes, needles, and used gloves in the appropriate containers.

Special Considerations

- If the patient has large, distended, highly visible veins, perform venipuncture without a tourniquet to minimize the risk of hematoma
- If the patient has a clotting disorder or is receiving anticoagulant therapy, maintain firm pressure on the venipuncture site for no less than 5 minutes after withdrawing the needle to prevent formation of a hematoma.

Blood processing

- Blood specimens will be stored in CRC refrigerator.
- All specimen's will be labeled with date, participant ID #, study title, visit number, and PI name
- Green top tube will be given to the CRC nurse for specimen requisition and sending to TVC laboratories for send out
- The other specimens will be storage on ice or at -4 °C until processed within 4-6 hours after collection within the Molecular Epidemiology Laboratory
- Serum will be collected after coagulation.
- Whole blood (EDTA tube) will be processed within 4-6 hours and separated into plasma, buffy coats (white cells).
- EDTA tube processing
 - Spin at 1500g for 10 min at 4°C
 - Transfer equal amounts of plasma to four 2ml tubes in sequential order
 - Transfer WBCs dropwise to two 2ml tubes
 - Wash remaining RBCs by adding 0.9% NaCl to bring total volume to ~10ml
 - Invert tube 6-8 time
 - Spin at 1500g for 10 min at 4°C
 - Suction off supernatant
 - Repeat wash, spin, and suction process
 - Transfer RBC to two 2ml tubes
 - Vials will be placed in specimen box for storage
 - Store tubes at -80°C
- Serum tube processing
 - Incubate at 37°C for 30 min

- Spin at 1500g for 10 min at 4°C
- 1-2 ml aliquots of the serum/plasma sample will be pipetted into each of 9-labeled cryovials.
- Transfer clot to one 3.5 ml tube
- Vials will be placed in specimen box for storage
- Store tubes at -80°C
- Vials will be placed in specimen box for storage
- Date, time and location of sample in the freezer will be recorded on the specimen log
- Blood specimens will be stored until use in relevant assays.

Blood sample allocation

- One aliquot of 200 µL of erythrocytes will be assayed for red blood cell phospholipid analysis in Dr. Larry Swifts Laboratory
- The remaining samples will be stored for additional assays in the Molecular Epidemiology core Laboratory freezer.

Protocol for urine sample collection

We will collect a total of 20 ml urine on each subject at each visit. Urine samples will be collected at study baseline (week 0), the study midpoint (week 2) and the end of the study (week 4). Collected urine will be used for the following study related measures:

- Urine tobacco metabolites: This will be a marker of exposure.
 - Urine volume required: 4ml
 - Specimen processing: see below
 - Laboratory location for storage:
 - Molecular Epidemiology Specimen Laboratory
MCN, B-2104, ext # 6-1351
 - Laboratory for specimen processing:
 - CRC Specimen Laboratory
MCN. Suite AA-3208
 - Laboratory for urine measurement:
TVC

Urine sample allocation

- Prior to long term storage in -80°C urine will be aliquoted into 7 3 ml cryovials for storage.
- Two cryovial will be sent for urine tobacco metabolites
- Urine will be stored on ice until processing
- Urine can be transported for laboratory testing at room temperature

10.0 Reporting Adverse Events

The potential risk to the subjects related to the drawing of venous blood and the use of fish oil supplements.

Sampling of venous blood includes risk such as bruising, bleeding, and infection. These complications are uncommon and phlebotomy is a routine part of general clinical care.

Omega-3 fatty acids have been part of the human diet for millennia and have uncommon and generally trivial side effects. In 1997 the Food and Drug Administration rules that an intake of up to 3 g/day of marine omega-3 fatty acids are Generally Recognized as Safe (GRAS)(135) (<http://www.epa.gov/fedrgstr/EPA-IMPACT/2002/February/Day-26/i4327.htm>). This ruling specifically considered the possible effects of fish oil on bleeding time, glycemic control, and LDL cholesterol. Fish oil supplements have been used in several large randomized controlled studies of cardiac patients.(136) In some trials doses as high as 12 g/d for durations of 2 years have been well tolerated.(137) In addition, fish oil supplements have been used in over X randomized controlled studies of pregnant women and are well tolerated. The most common side effect of fish oil is a fishy

after-taste which is less of a problem with pharmaceutical grade supplements. Minor gastrointestinal symptoms occur in 5 percent of patients. (135) Fish oil has been approved by the FDA for treatment of hypertriglyceridemia.

10.1 Adverse Events

Adverse event (AE) grading and attribution scale

For the purposes of this study and adverse event is defined as any untoward medical occurrence in a subject, not necessarily having a causal relationship with the study. AE's are graded as Mild, Moderate, Severe, Life-threatening, and Death and are attributed according to the relationship to the study drug and/or procedure as Not related, Unlikely, Possible, Probable, or Definite.

All AEs that occur after the informed consent is signed must be recorded on the AE CRF (**Appendix G: Adverse Events**) whether or not related to study agent.

Data elements to be collected for AE reporting include:

- 1) Event description
- 2) Event onset date and event end time
- 3) Severity grade
- 4) Attribution to study agent
- 5) Whether or not the report was reported as a SAE
- 6) Whether or not the participant dropped out of the study due to the event
- 7) Outcome of the event

Adverse Event Severity Grading Guidelines

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observation only; intervention no indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL) ¹
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limited self-care ADLs ²
4	Life-threatening	Life-threatening consequences; urgent intervention indicated
5	Fatal	Death related to AE

¹Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money etc.

²Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden

Adverse Event Attribution Guidelines

The possibility that the AE is related to the study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

10.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that

- 1) Results in death
- 2) Is life-threatening
- 3) Requires inpatient hospitalization or prolongation of existing hospitalization

- 4) Results in persistent or significant disability/incapacity
- 5) Is a congenital anomaly/birth defect.

Plan for unanticipated AE reporting

SAEs and unanticipated AEs will be reported immediately by telephone to the Vanderbilt University IRB (Chasiety Turner HS#1). An Adverse Event Report form will be completed and returned to the VU IRB within 2 working days.

Plan for annual reporting of AEs

Annual reports are submitted to the VU IRBs and will contain a) the number of adverse events and an explanation of how each event was handled, b) the number of complaints and how each complaint was handled, c) the number of subject withdrawals and an explanation of why the subject withdrew or was withdrawn, and d) the number of protocol violations and how each was handled.

11.0 Study Monitoring

11.1 Data Management and Security

Confidentiality and ethical considerations will be addressed as follows. All identifying documents, data and specimens collected as a result of this study will be retained by the investigator. Access to this material will be available only to the research investigator and his staff. Paper (hard) copies of study documents will be kept in a locked file cabinet. Electronic copies of documents will be stored in a password protected database on a secured server. If results of this study are to be published, only code numbers will be used for identification. Participants will not be identified by name.

11.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRFs) in REDCap. All CRFs will be approved by the Vanderbilt IRB prior to use. Study staff will enter data into the electronic research record either directly. The primary source of data collection and storage will be REDCap. REDCap offers a secure, password-protected web-based record..

11.3 Source Documents

All source documents will be collected and stored in a locked file cabinet within the Division of General Internal Medicine and Public Health offices. Only study personnel will have access to these files.

11.4 Record Retention

Hard copies of all records will be stored in locked filing cabinets located in locked office space. Electronic databases are located in restricted access folders within the Vanderbilt University Medical Center computer network. Access to the database is restricted by password or permission-based access. All identifying information will be removed and/or deleted from the participant records at 2 years following the conclusion of the investigation. Biological samples are coded and retained in a locked location with access limited to specific study personnel. Source documents and data will be stored in a secure facility in compliance with the Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP) and the Vanderbilt IRB regulations and guidelines.

12.0 Statistical Considerations

This study will provide feasibility data regarding the recruitment strategy, the acceptability of n-3 LCPUFAs supplementation in pregnant women, and preliminary data on effect size for power estimation. Our study power is based on our primary outcome, reduction of cigarettes per day at 4 weeks.

12.1 Study Design/Description

The study is a randomized, double-blind, placebo-controlled, parallel arm trial design. The two arms include one treatment arm and one placebo arm. Treatment will be 4.2 grams/day of EPA + DHA. Placebo will be 5 grams of olive oil in capsule form. The intervention period will last 4-weeks. We will randomize 40 participants into the two treatment arms. As this is a feasibility study, we have not powered the study to account for drop-outs

12.2 Randomization

The study includes two groups and randomization in permuted blocks. Randomization will be stratified based on daily cigarette use (≤ 5 cigarettes per day/ > 5 cigarettes per day).

12.3 Accrual and Feasibility

Approximately 400 women delivered at VUMC last year who were active smokers. With an average of 30 pregnant smokers delivering at Vanderbilt per month we would estimate an accrual rate of 6-8 subjects a month reaching our study estimate of 40 subjects in 6 months.

12.4 Primary Objective, Endpoint, Analysis Plan

Descriptive statistics, including means, standard deviation, and ranges for continuous variables, as well as percent and frequencies for categorical variables, will be presented. Standard graphing and screening techniques will be used to detect outliers and to ensure data accuracy. For the primary analysis of post-intervention cigarettes per day, we will perform a Wilcoxon (Mann Whitney) rank sum test evaluated at a 5% significance level. This data analysis plan will be carried out using statistical software SAS® (Cary, North Carolina) or statistical package R (R Development Core Team, 2008).

For our primary outcome we will compare the change in total number of cigarettes smoked per day from baseline to 4-weeks. We will compare the change outcome at 4-weeks between arms using logistic regression using repeated measured ANOVA.

For our primary outcome, if we assume a baseline number of cigarettes per day of 11 with a SD of 5(34), we should be able to detect a reduction by 41% in cigarettes per day in smokers allocated to the n-3 LCPUFA arm compared to placebo with 80% power.

12.5 Secondary Objective, Endpoint(s), Analysis Plan

Secondary outcomes include: change in the Fagerström Tolerance Questionnaire and point prevalence abstinence at 4 weeks biochemically confirmed by end-expired carbon monoxide. We will compare the change outcome at 4-weeks between arms using logistic regression using repeated measured ANOVA.

The Fagerström Tolerance Questionnaire is on a 10-point Likert scale. Estimating a baseline score of $7.3 \pm 1.6(35)$ we have 80% power to detect a difference of 1 SD between the study arms.

A goal of this pilot study is to estimate potential effect sizes for a future R01 study. We are underpowered to detect a main effect however the study will give us an effect size from which to power a larger study. No prior studies exists which have used n-3 LCPUFA supplementation in pregnant women who are interested in quitting. We estimate a 50% abstinence rate in all comers. With 20 subjects per arm we would only have 24% power to identify a 50% increase in abstinence rates assuming a two-tailed type 1 error rate of 0.05.

12.6 Evaluation of Toxicity

All participants will be evaluated for toxicity from the time of their first dose of study agent

12.7 Evaluation of Response

All participants included in the study will be assessed for response to intervention, even if there are major protocol deviations.

All of the participants who met the eligibility criteria and receive the study agent will be included in the intention-to-treat analysis.

13.0 Ethical and Regulatory Considerations

13.1 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators will have obtained written approval to conduct the study from the Vanderbilt IRB. Should changes to the study become necessary, protocol amendment will be submitted according to VUMC guidelines.

13.2 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. The Investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, she will be asked to sign and date the Informed Consent document. The study agent will not be released to a participant who has not signed the Informed Consent document. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

13.3 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practices (GCP) and the applicable regulatory requirements

13.4 Financing, Expenses, and/or Insurance

Participants will be compensated \$225 for participation (i.e. Clinic Visit 1 \$75, Clinic Visit 2 \$75, Clinic Visit 3 \$75) if all the procedures are completed. This is to compensate for any financial cost due to travel and the inconvenience of blood draws. The participant compensation plan will be pro-rated based on the number of study visits completed. Participants who complete the entire study will receive a total of \$225. Participants will sign the Reimbursement Form (**Appendix B: Study Visit Forms**) which will be submitted to the Division of General Internal Medicine and Public Health for processing. Subjects will be told to expect at least 2-4 weeks for payment.

14.0 Literature Cited:

1. Jha P, Ramasundarahettige C, Landsman V, Rostron B, Thun M, Anderson RN, et al. 21st-century hazards of smoking and benefits of cessation in the United States. *N Engl J Med*. 2013;368(4):341-50.
2. Ellard GA, Johnstone FD, Prescott RJ, Ji-Xian W, Jian-Hua M. Smoking during pregnancy: the dose dependence of birthweight deficits. *Br J Obstet Gynaecol*. 1996;103(8):806-13.
3. Li CQ, Windsor RA, Perkins L, Goldenberg RL, Lowe JB. The impact on infant birth weight and gestational age of cotinine-validated smoking reduction during pregnancy. *JAMA*. 1993;269(12):1519-24.
4. Mitchell EA, Tuohy PG, Brunt JM, Thompson JM, Clements MS, Stewart AW, et al. Risk factors for sudden infant death syndrome following the prevention campaign in New Zealand: a prospective study. *Pediatrics*. 1997;100(5):835-40.
5. Pineles BL, Hsu S, Park E, Samet JM. Systematic Review and Meta-Analyses of Perinatal Death and Maternal Exposure to Tobacco Smoke During Pregnancy. *Am J Epidemiol*. 2016;184(2):87-97.
6. Curtin SC, Mathews, T.J. Smoking prevalence and cessation before and during pregnancy: data from the birth certificate, 2014. *Natl Vital Stat Rep*. 2016;65(1).
7. Tong VT, Dietz, P.M., Morrow, B., D'Angelo, D., V., Farr, S. L., Rockhill, K.M., England, L.J. Trends in smoking before, during, and after pregnancy-pregnancy risk assessment monitoring system, United States, 40 sites, 2000-2010. *MMWR Morb Mortal Wkly Rep*. 2013;62(SS06):1-19.
8. Alves E, Azevedo A, Correia S, Barros H. Long-term maintenance of smoking cessation in pregnancy: an analysis of the birth cohort generation XXI. *Nicotine Tob Res*. 2013;15(9):1598-607.
9. Calder PC. N-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. *Lipids*. 2003;38(4):343-52.
10. Harris WS, Pottala JV, Lacey SM, Vasan RS, Larson MG, Robins SJ. Clinical correlates and heritability of erythrocyte eicosapentaenoic and docosahexaenoic acid content in the Framingham Heart Study. *Atherosclerosis*. 2012;225(2):425-31.
11. Hibbeln JR, Makino KK, Martin CE, Dickerson F, Boronow J, Fenton WS. Smoking, gender, and dietary influences on erythrocyte essential fatty acid composition among patients with schizophrenia or schizoaffective disorder. *Biol Psychiatry*. 2003;53(5):431-41.
12. Nosova EV, Chong KC, Alley HF, Harris WS, Boscardin WJ, Conte MS, et al. Clinical correlates of red blood cell omega-3 fatty acid content in male veterans with peripheral arterial disease. *J Vasc Surg*. 2014;60(5):1325-31.
13. Simon JA, Fong J, Bernert JT, Jr., Browner WS. Relation of smoking and alcohol consumption to serum fatty acids. *Am J Epidemiol*. 1996;144(4):325-34.
14. Murff HJ, Tindle HA, Shrubsole MJ, Cai Q, Smalley W, Milne GL, et al. Smoking and red blood cell phospholipid membrane fatty acids. *Prostaglandins Leukot Essent Fatty Acids*. 2016;112:24-31.
15. Chalon S, Vancassel S, Zimmer L, Guilloteau D, Durand G. Polyunsaturated fatty acids and cerebral function: focus on monoaminergic neurotransmission. *Lipids*. 2001;36(9):937-44.
16. Zimmer L, Hembert S, Durand G, Breton P, Guilloteau D, Besnard JC, et al. Chronic n-3 polyunsaturated fatty acid diet-deficiency acts on dopamine metabolism in the rat frontal cortex: a microdialysis study. *Neurosci Lett*. 1998;240(3):177-81.
17. Zimmer L, Delpal S, Guilloteau D, Aioun J, Durand G, Chalon S. Chronic n-3 polyunsaturated fatty acid deficiency alters dopamine vesicle density in the rat frontal cortex. *Neurosci Lett*. 2000;284(1-2):25-8.
18. Chalon S. Omega-3 fatty acids and monoamine neurotransmission. *Prostaglandins Leukot Essent Fatty Acids*. 2006;75(4-5):259-69.
19. Zimmer L, Vancassel S, Cantagrel S, Breton P, Delamanche S, Guilloteau D, et al. The dopamine mesocorticolimbic pathway is affected by deficiency in n-3 polyunsaturated fatty acids. *Am J Clin Nutr*. 2002;75(4):662-7.
20. Ahmad SO, Park JH, Radel JD, Levant B. Reduced numbers of dopamine neurons in the substantia nigra pars compacta and ventral tegmental area of rats fed an n-3 polyunsaturated fatty acid-deficient diet: a stereological study. *Neurosci Lett*. 2008;438(3):303-7.

21. Zaniowska M, Przegalinski E, Filip M. Nicotine dependence - human and animal studies, current pharmacotherapies and future perspectives. *Pharmacol Rep.* 2009;61(6):957-65.
22. Mansvelder HD, McGehee DS. Cellular and synaptic mechanisms of nicotine addiction. *J Neurobiol.* 2002;53(4):606-17.
23. Zaparoli JX, Galduroz JC. Treatment for tobacco smoking: a new alternative? *Med Hypotheses.* 2012;79(6):867-8.
24. Rabinovitz S. Effects of omega-3 fatty acids on tobacco craving in cigarette smokers: A double-blind, randomized, placebo-controlled pilot study. *J Psychopharmacol.* 2014;28(8):804-9.
25. Zaparoli JX, Sugawara EK, de Souza AA, Tufik S, Galduroz JC. Omega-3 Levels and Nicotine Dependence: A Cross-Sectional Study and Clinical Trial. *Eur Addict Res.* 2016;22(3):153-62.
26. Gould JF, Smithers LG, Makrides M. The effect of maternal omega-3 (n-3) LCPUFA supplementation during pregnancy on early childhood cognitive and visual development: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2013;97(3):531-44.
27. Saccone G, Berghella V. Omega-3 long chain polyunsaturated fatty acids to prevent preterm birth: a systematic review and meta-analysis. *Obstet Gynecol.* 2015;125(3):663-72.
28. Makrides M, Duley L, Olsen SF. Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction. *The Cochrane database of systematic reviews.* 2006(3):CD003402.
29. Koletzko B, Cetin I, Brenna JT, Perinatal Lipid Intake Working G, Child Health F, Diabetic Pregnancy Study G, et al. Dietary fat intakes for pregnant and lactating women. *Br J Nutr.* 2007;98(5):873-7.
30. Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, Cetin I, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. *J Perinat Med.* 2008;36(1):5-14.
31. Shin A, Shrubsole MJ, Ness RM, Wu H, Sinha R, Smalley WE, et al. Meat and meat-mutagen intake, doneness preference and the risk of colorectal polyps: the Tennessee Colorectal Polyp Study. *Int J Cancer.* 2007;121(1):136-42.
32. Shrubsole MJ, Wu H, Ness RM, Shyr Y, Smalley WE, Zheng W. Alcohol drinking, cigarette smoking, and risk of colorectal adenomatous and hyperplastic polyps. *Am J Epidemiol.* 2008;167(9):1050-8.
33. Murff HJ, Shrubsole MJ, Chen Z, Smalley WE, Chen H, Shyr Y, et al. Nonsteroidal anti-inflammatory drug use and risk of adenomatous and hyperplastic polyps. *Cancer Prev Res (Phila).* 2011;4(11):1799-807.
34. Anti M, Armelao F, Marra G, Percesepe A, Bartoli GM, Palozza P, et al. Effects of different doses of fish oil on rectal cell proliferation in patients with sporadic colonic adenomas. *Gastroenterology.* 1994;107(6):1709-18.
35. Anti M, Marra G, Armelao F, Bartoli GM, Ficarelli R, Percesepe A, et al. Effect of omega-3 fatty acids on rectal mucosal cell proliferation in subjects at risk for colon cancer. *Gastroenterology.* 1992;103(3):883-91.
36. Bartoli GM, Palozza P, Marra G, Armelao F, Franceschelli P, Luberto C, et al. n-3 PUFA and alpha-tocopherol control of tumor cell proliferation. *Mol Aspects Med.* 1993;14(3):247-52.
37. Donny EC, Denlinger RL, Tidey JW, Koopmeiners JS, Benowitz NL, Vandrey RG, et al. Randomized Trial of Reduced-Nicotine Standards for Cigarettes. *N Engl J Med.* 2015;373(14):1340-9.
38. Pollak KI, Oncken CA, Lipkus IM, Lyna P, Swamy GK, Pletsch PK, et al. Nicotine replacement and behavioral therapy for smoking cessation in pregnancy. *Am J Prev Med.* 2007;33(4):297-305.
39. Hurt RD, Sachs DP, Glover ED, Offord KP, Johnston JA, Dale LC, et al. A comparison of sustained-release bupropion and placebo for smoking cessation. *N Engl J Med.* 1997;337(17):1195-202.